Early and Delayed Shut-off of Host Protein Synthesis in Cells Infected with Herpes Simplex Virus

(Accepted 25 February 1982)

SUMMARY

A mutant of herpes simplex virus type 1 [HSV-1(HFEM)], tsB7, appears to have two temperature-sensitive functions. One is required during the first hour of infecting a cell (suggesting that it is performed by a virion protein) and the other is the non-essential function of "early shut-off" of cellular protein synthesis, which is mediated by a virion protein. The latter function remained temperature-sensitive in a revertant virus (RC2) grown at the non-permissive temperature (39 °C). However, under these conditions RC2 did cause inhibition of host synthesis, showing that "delayed shut-off", requiring virus protein synthesis, can occur independently of early shut-off, which is mediated by a virion protein. Early shut-off by u.v.-irradiated tsB7 was reversed when the temperature was raised, whereas delayed shut-off by intact tsB7 was not. Of two wild-type strains of virus examined, HSV-1(F) also exhibited temperature-sensitive early shut-off, but HSV-2(G) did not.

After infection with herpes simplex virus (HSV) cellular protein synthesis declines and polysomes becomes disaggregated (Sydiskis & Roizman, 1966), apparently as a result of the action of some component of the infecting virions (Fenwick & Walker, 1978; Nishioka & Silverstein, 1978). In some cases, however, it has been found that the suppression of host protein synthesis is associated with the synthesis of virus proteins, in particular β-proteins (Honess & Roizman, 1974).

A temperature-sensitive mutant of HSV-1, tsB7, failed to inhibit protein synthesis at the non-permissive shut-off (Knipe et al., 1981). However, as it was also unable to make any detectable virus proteins at this temperature, it was not clear whether the protein that was responsible for suppressing host cell protein synthesis at the permissive temperature was itself temperature-sensitive or whether the lack of suppression was a secondary consequence of a block in a very early temperature-sensitive step, such as uncoating of the virus DNA.

Our experiments with tsB7 and a temperature-resistant revertant virus (RC2) suggest that tsB7 directs the production of two temperature-sensitive proteins: a virion protein that causes early suppression of host synthesis, a function that is not essential for virus growth, and the protein that controls a very early essential step in infection. They also confirm that there are two independent mechanisms of attack on cellular protein synthesis: "early shut-off" by a virion component and "delayed shut-off" which requires virus protein synthesis.

Monolayers of Vero cells in 25 cm² tissue culture flasks were infected at 20 °C at a multiplicity of infection of 10 to 20. At zero time (after an adsorption period of 20 min) they were immersed in water-baths at 34 or 39 °C. Details of cells, virus strains and techniques of assay, infection, labelling with ¹⁴C-amino acids, electrophoresis in polyacrylamide gradient gels containing SDS, and u.v. irradiation of virus (with a dose of 2 × 10⁻⁴ J/mm²) were as described by Fenwick & Walker (1978).

The temperature-sensitive mutant virus, tsB7, was kindly provided by Dr A. Buchan, University of Birmingham, U.K. It was obtained by growing HSV-1(HFEM) in the presence of bromodeoxyuridine. Its efficiency of plating at 39 °C relative to that at 34 °C was 6 × 10⁻⁵.
The temperature-resistant variant, RC2, was isolated by inoculating monolayers of $4 \times 10^5$ Vero cells in the wells of a tissue culture tray with $5 \times 10^4$ p.f.u. (titre at $34^\circ C$) of tsB7. The contents of a well in which a single plaque developed during 3 days at $39^\circ C$ were disrupted by ultrasonication and used to infect a $25 \text{ cm}^2$ culture of cells which was further incubated at $39^\circ C$. The yield from this culture was used as seed for subsequent infections, which were incubated at $37^\circ C$. The virus preparation used in these experiments had a titre of $4 \times 10^9$ p.f.u./ml at $34$ or $39^\circ C$. Other preparations ranged from 0·5 to 0·01 in relative efficiency of plating at $39$ and $34^\circ C$, although they were all able, unlike tsB7, to initiate virus protein synthesis at $39^\circ C$.

At its permissive temperature ($33$ to $34^\circ C$) tsB7 induces virus-specific protein synthesis and suppresses the production of cellular proteins, but at $39^\circ C$ it does neither (Knipe et al., 1981). We observed that if the temperature of infected cultures was raised from $34$ to $39^\circ C$ as early as 45 min after infection, the synthesis of virus proteins could be detected by electrophoresis and autoradiography after labelling between 3 and 4 h. The essential temperature-sensitive function is evidently needed only during the first hour of infection.

Infection with tsB7 that had been irradiated with u.v. light resulted in substantial suppression of host protein synthesis within 4 h at $34^\circ C$, although no virus-specific protein synthesis was detected. If the temperature was raised to $39^\circ C$ at 45 min (having allowed time for the infection to proceed beyond the temperature-sensitive block) host protein synthesis was not inhibited. This suggests that shut-off was caused by a temperature-sensitive virion protein independently of the conditional-lethal mutation of tsB7. We have used the term 'early shut-off' to describe this virion-mediated inhibition.

Early shut-off by tsB7 was found to be reversible. When cells that had been suppressed by u.v.-inactivated tsB7 at $34^\circ C$ were then incubated at $39^\circ C$, the rate of protein synthesis was restored to normal, while cells kept at $34^\circ C$ remained suppressed. In contrast, if cells infected with intact tsB7 at $34^\circ C$ were raised to $39^\circ C$ at 3 h, cellular protein synthesis remained inhibited, indicating that a further, non-reversible, stage had been reached, presumably as a result of virus gene expression. We have called this process 'delayed shut-off' to distinguish it from early shut-off, which is caused by inactivated virus.

In order to confirm that early shut-off is controlled independently of the conditional-lethal mutation of tsB7, a temperature-resistant revertant was isolated. Fig. 1 shows the results of infection with tsB7 or revertant RC2. Whereas tsB7 is prevented from initiating virus protein synthesis or suppressing host synthesis at $39^\circ C$ (compare lanes 1 and 4), RC2 can do both (lanes 3 and 6). However, infection with u.v.-inactivated RC2, or with intact RC2 in the presence of actinomycin to prevent gene expression, revealed that early shut-off was still temperature-sensitive (Fig. 2, lanes 8 and 10, compare lanes 3 and 5). We conclude that early shut-off is controlled by a temperature-sensitive virion protein in tsB7 and RC2, and is not an essential function for virus growth. On the other hand, suppression did occur when cells were infected with intact RC2 at $39^\circ C$ (Fig. 2, lane 7); thus, it appears that delayed shut-off (requiring virus protein synthesis) can occur independently of early shut-off.

Having observed that the temperature sensitivity of early shut-off by tsB7 was not due to a conditional-lethal mutation, two other unrelated strains of virus were examined to see whether this function was generally temperature-sensitive. Experiments with u.v.-inactivated viruses showed that the early shut-off protein of HSV-1(F) is temperature-sensitive but that of HSV-2(G) is not. HSV-1(F) is able to make a range of early proteins at $39^\circ C$, although it is not able to form plaques. Thus, like tsB7, it appears to have a second temperature-sensitive function, possibly that of an $\alpha$-polypeptide (Knipe et al., 1981).

The observation that some strains of herpesvirus possess a temperature-sensitive virion protein that is responsible for the early suppression of protein synthesis in infected cells has made clear the distinction between this process, 'early shut-off', and 'delayed shut-off',
Fig. 1. Cells infected with tsB7 (lanes 1 and 4), or revertant RC2 (lanes 3 and 6) or mock-infected cells (lanes 2 and 5) were incubated from zero time at 34 °C (lanes 1 to 3) or 39 °C (lanes 4 to 6) and labelled with 14C-amino acids at the same temperatures from 6 to 7 h post-infection. Polypeptides in cell lysates were separated by electrophoresis in polyacrylamide gel and an autoradiogram prepared from the dried gel. Infected cell polypeptides (shown on the right-hand side of the gel) are numbered according to Morse et al. (1978).

which is not temperature-sensitive and depends on virus protein synthesis. The two processes may well correspond to dissociation of polysomes and degradation of cellular mRNA respectively. Cellular mRNA is degraded after infection with intact virus (Nishioka & Silverstein, 1978; Inglis & Newton, 1981) but not with u.v.-inactivated virus. Therefore, it has been proposed (Nishioka & Silverstein, 1978) that the dissociation of ribosomes from host mRNA is caused by a constituent of the ingoing virus particles but that the destruction of host mRNA depends on expression of virus genes.

It has been observed (Powell & Courtney, 1975; Pereira et al., 1977; Morse et al., 1978) that some strains of HSV-2 suppress host protein synthesis more rapidly than HSV-1 strains, suggesting the possibility that early shut-off, as defined here, is characteristic of HSV-2. Such a generalization cannot be made, however, since HSV-1(F) and HSV-1(HFEM) tsB7 do cause early shut-off in Vero cells, while HSV-2(HG52) (Timbury, 1971) does not (M. Fenwick & J. Clark, unpublished results). Of course, factors such as type of host cell and particle to p.f.u. ratio in the virus preparation may also influence the rate of early shut-off.

The mutant tsB7 appears to direct the synthesis of two temperature-sensitive proteins. One is required at a very early stage in infection and the other is a virion protein performing the non-essential function of early shut-off. The essential protein was restored to normal
temperature insensitivity in the revertant virus, enabling it to grow at 39 °C and suppress host protein synthesis (late shut-off) but the virion inhibitor remained temperature-sensitive. The nature of the early temperature-sensitive essential function of tsB7 is not known. In unpublished experiments we were unable to detect the synthesis of α-proteins after removal of cycloheximide from cells infected in its presence and maintained at 39 °C for 5 h. Thus, it appears that the synthesis of virus α-mRNA is not initiated at 39 °C, although both synthesis and translation of mRNA can occur at 39 °C after a short period at 34 °C. It seems likely that another virion protein is involved. It has been suggested that a defect in transcription of α-genes or in uncoating of the virus DNA might account for this early block (Roizman et al., 1981; Knipe et al., 1981).

The conditional-lethal mutation of tsB7 has been located between positions 0.46 and 0.52 on the genetic map by marker rescue experiments (Knipe et al., 1981). The map position of the gene controlling early shut-off was estimated at 0.52 to 0.59 by studying recombinants between strains of HSV-1 and HSV-2 which differed in their rates of early shut-off, measured in the presence of actinomycin (Fenwick et al., 1979). Although the mapping data do not exclude the possibility (pointed out by Knipe et al., 1981) that the conditional-lethal mutation could be in the early shut-off gene, our experiments support the idea that two separate genes
Short communications

are involved and that the failure of early shut-off by tsB7 at 39 °C is not a secondary consequence of the conditional-lethal mutation.

This work was supported in part by a grant to Professor H. Harris from the Cancer Research Campaign.

Sir William Dunn School of Pathology
University of Oxford
South Parks Road, Oxford OX1 3RE, U.K.

M. L. FENWICK*  
JANE CLARK

REFERENCES


(Received 23 November 1981)